Draft Implementation Plan
for the 2008-2012 NICEATM-ICCVAM Five Year Plan
June 2009

Interagency Coordinating Committee on the
Validation of Alternative Methods

National Toxicology Program Interagency Center for the
Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

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Executive Summary

In 2008, the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) developed and published a five-year plan in conjunction with Federal agency program offices. The plan describes how NICEATM and ICCVAM will foster and promote research, development, translation, validation, and regulatory acceptance of alternative test methods that reduce, refine, and replace the use of animals for safety testing, while maintaining and promoting scientific quality and protecting the health of people, animals, and the environment.

This working document describes how NICEATM and ICCVAM are implementing the strategies outlined in the five-year plan. Implementation activities address the four key challenges in the five-year plan:

1. Conducting and facilitating alternative test method activities in priority areas
2. Identifying and promoting research initiatives that are expected to support the future development of innovative alternative test methods
3. Fostering the acceptance and appropriate use of alternative test methods
4. Developing partnerships and strengthening interactions with ICCVAM stakeholders in order to facilitate meaningful progress

Conducting and Facilitating Alternative Test Method Activities in Priority Areas

ICCVAM priorities emphasize alternatives for those regulatory test methods that can involve significant animal pain and distress and that can involve large numbers of animals. Currently, the four highest-priority testing areas are biologics, ocular toxicity, dermal toxicity, and acute toxicity. NICEATM and ICCVAM will identify critical knowledge and data gaps that must be addressed in order to advance alternative methods for these and other evolving priority areas (e.g., immunotoxicity, reproductive and developmental toxicity, the safety assessment of manufactured nanomaterials). ICCVAM will involve regulatory agencies, the scientific community, and other stakeholders in these activities. ICCVAM will distribute recommendations to stakeholder organizations with resources to carry out the recommended research, development and validation activities. ICCVAM and NICEATM will interact with participating stakeholders throughout the process to help develop methods that are useful for regulatory testing. Upon receiving validation study results, ICCVAM will evaluate the scientific validity of methods for regulatory testing purposes and provide recommendations to regulatory agencies on demonstrated usefulness and limitations.

Identifying and Promoting New Science and Technology

NICEATM and ICCVAM are working with Federal agencies and other stakeholders to link their research and development activities to the standardization and validation of alternative test methods. ICCVAM agencies have been asked to describe any ongoing and planned research, development, translation, and validation activities relevant to test methods that reduce, refine, and replace the use of animals. As part of this implementation plan, the role of ICCVAM working groups will be expanded to include consultation with test method developers to help

ensure that test methods are designed to meet regulatory needs. This consultation will also help optimize validation studies necessary to determine their usefulness and limitations for regulatory decision-making.

ICCVAM has established a Research and Development Working Group (RDWG) to help ICCVAM implement activities relevant to incorporating new science and technology. The RDWG is specifically charged with helping NICEATM and ICCVAM identify and promote research that incorporates new technologies expected to support the future development of new test methods and approaches that will reduce, refine, and replace animal use in toxicity testing. The RDWG will help identify test methods in the development phase that would benefit from referral and interactions with an ICCVAM test method working group.

Fostering Regulatory Acceptance and Appropriate Use of Alternative Methods

Once regulatory authorities have accepted an alternative test method, ICCVAM will promote its use by communicating the outcomes of ICCVAM review activities and/or workshops in the *Federal Register* and peer-reviewed journals and at training courses and national and international scientific meetings. Emphasis will be placed on informing the scientific community, including Institutional Animal Care and Use Committees, of new alternatives that should be considered in order to ensure compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and Animal Welfare Act regulations, which require consideration of such methods before testing is conducted in animals.

ICCVAM will cosponsor workshops with government and nongovernment organizations, where appropriate. These workshops will 1) evaluate the state of the science related to the development and validation of alternative test methods and 2) identify high-priority research, development, translation, and validation activities necessary to advance and characterize the usefulness of such methods. The workshop results will be broadly communicated to individuals and organizations that conduct these activities.

Developing Partnerships and Strengthening Interactions with ICCVAM Stakeholders

ICCVAM will foster international collaboration by including experts from the international scientific community in workshops that review the state of the science for particular test method areas. Where appropriate, NICEATM and ICCVAM will also invite representatives from international organizations such as the Organisation for Economic Co-operation and Development (OECD) and from OECD member countries to attend and participate in relevant NICEATM and ICCVAM-sponsored workshops, peer reviews, and other scientific activities. Similarly, to further ensure the development of scientifically valid international test guidelines, NICEATM and ICCVAM will encourage participation of their scientists in U.S. delegations to OECD test guideline meetings, expert consultations, and workshops.

Introduction

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is an interagency committee created by the National Institute of Environmental Health Sciences (NIEHS) in 1997 and established as a permanent committee by the ICCVAM Authorization Act of 2000. Administered by NIEHS under the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), ICCVAM is composed of members from 15 Federal agencies. The committee's mission is to facilitate development, validation, and regulatory acceptance of new, revised, and alternative test methods that reduce, refine, and replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment.

An overall goal is for ICCVAM to assume a greater leadership role in promoting research, development, translation, validation, and regulatory acceptance of alternative test methods. NICEATM and ICCVAM developed a Five-Year Plan that builds on the ICCVAM mission, vision, and strategic priorities to achieve progress and to inform the public of their plans and approaches.[1] To implement this plan, NICEATM and ICCVAM will work with a broad range of stakeholders, including Federal agencies, national and international validation and test guideline organizations, industry, academia, and the animal welfare community. Success will depend on these interactions both within and outside of ICCVAM agencies. ICCVAM will take a proactive leadership role and identify and develop collaborations that will include experienced scientists that can bring state-of-the-art science to the forefront.

ICCVAM, as an interagency committee, does not have resources to conduct research, development, and validation studies. Rather, it depends on its many stakeholders to conduct and achieve successful test method research, development, translation, and validation efforts. Many Federal agencies and other organizations conduct research that could ultimately result in the development and validation of an alternative test method for regulatory use. These test methods can then be evaluated by ICCVAM for potential regulatory use.

ICCVAM's priorities are based on agency priorities[2] as well as other criteria[3] that include:

- The potential impact that alternative test methods may have on reducing, refining, or replacing the use of animals for testing, taking into consideration the severity of pain and distress and numbers of animals involved
- The potential for the proposed test method(s) to better predict adverse health or environmental effects
- The applicability of testing alternatives across agencies

ICCVAM uses these criteria to prioritize test method nominations and submissions for evaluation.

Several Federal agencies are responsible for safeguarding human and animal health and the environment. To assess health and environmental risks, Federal agencies have developed and adopted testing methods to evaluate the potential hazards or safety of chemicals, and other

[1] http://iccvam.niehs.nih.gov/docs/5yearplan.htm
[2] Testing priorities of individual Federal agencies may differ because of the different statutory mandates under which they operate.
[3] http://iccvam.niehs.nih.gov/SuppDocs/submission.htm

products. However, new and revised toxicological test methods are being developed with increasing frequency as scientists seek to incorporate new science and technology. ICCVAM and NICEATM serve a unique role in helping to evaluate the usefulness and limitations of these methods and achieving the acceptance of those found to be scientifically valid for regulatory purposes. This interagency cooperation provides an efficient and effective mechanism for Federal test method review and helps to ensure that new and revised test methods meet the needs of Federal agencies while reducing, refining and replacing the use of animals in testing where scientifically feasible.

In implementing the strategies outlined in the Five-Year Plan, NICEATM and ICCVAM will address four key challenges: 1) conducting and facilitating activities in priority areas, 2) identifying and promoting research initiatives that are expected to support the future development of innovative alternative test methods, 3) fostering the acceptance and appropriate use of alternative test methods through outreach and communication, and 4) developing partnerships and strengthening interactions with ICCVAM stakeholders in order to facilitate meaningful progress. While ICCVAM has accomplished much during its first 10 years, this document focuses on the plans to implement the goals and objectives set forth for the next 5 years.

Challenge #1: Conduct and Facilitate Alternative Test Method Activities In Priority Areas

ICCVAM priorities emphasize alternatives for those regulatory test methods that can use large numbers of animals and that can involve significant animal pain and distress. Currently, the four highest-priority testing areas are biologics, ocular toxicity, dermal toxicity, and acute toxicity. Other priorities include test methods for immunotoxicity, endocrine disruptor effects, pyrogenicity, reproductive and developmental toxicity, chronic toxicity and carcinogenicity, and the safety assessment of manufactured nanomaterials.

NICEATM and ICCVAM will continue to promote research, development, translation, and validation of alternative test methods by identifying critical knowledge and data gaps that need to be addressed in order to advance alternative methods for these and other evolving priority areas. ICCVAM will involve regulatory agencies, the scientific community, and other stakeholders in these activities, and distribute recommendations to stakeholder organizations with resources to carry out the recommended research, development and validation activities. ICCVAM and NICEATM will interact with stakeholders during the R&D process as well as during validation studies to facilitate the development of methods that are useful for regulatory testing purposes. Following receipt of validation studies, ICCVAM will evaluate the scientific validity of methods for regulatory testing purposes and provide recommendations to regulatory agencies on demonstrated usefulness and limitations.

Biologics Testing

Goal

Identify and promote research, development, translation, and validation activities for priority test methods that may further reduce, refine, and replace animals in regulatory testing for biologics.

Specific Objectives

- Recommend how *in vitro* test systems and earlier more humane endpoints can be used to further reduce, refine, and eventually replace animal use for vaccine potency and efficacy testing while ensuring the protection of human and animal health.
- Identify and prioritize future research initiatives necessary to advance development and validation of *in vitro* methods for vaccine potency and efficacy testing.
- Discuss how to promote the collection and submission of *in vitro* and *in vivo* test data in order to advance the development and validation of more predictive *in vitro* test methods and earlier more humane endpoints for vaccine potency testing.

Planned Activities for Implementation

1. Reactivate the ICCVAM Biologics Working Group (BWG)
    - ICCVAM agencies will be asked to nominate new members with expertise specific to vaccine potency (BWG previously constituted specifically to evaluate botulinum neurotoxin potency testing methods).
    - Charge the BWG with developing a scientific workshop to 1) evaluate the state of the science for possible alternatives and 2) the use of humane endpoints in *in vivo* potency tests. Goals of the workshop include:

- Recommend how *in vitro* test systems and earlier more humane endpoints can be used to further reduce, refine, and eventually replace animal use for vaccine potency testing while ensuring the protection of human and animal health.
- Identify and prioritize future research initiatives necessary to advance development and validation of *in vitro* methods for vaccine potency testing.
- Discuss how to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods and earlier more humane endpoints for vaccine potency testing.

Timeframe: BWG to begin planning in 2009; workshop convened in 2010

2. Evaluate in *vitro* Potency Tests for Leptospirosis vaccines being developed by the U.S. Department of Agriculture (USDA).
    - Obtain data from an ongoing USDA/University of Michigan, Michigan State University validation study on in *vitro* potency tests for Leptospirosis vaccines.
    - Conduct a formal evaluation of the usefulness and limitations of the test methods once the validation study is complete and submitted to ICCVAM.

Timeframe: Draft ICCVAM test method recommendations to be released to an independent expert peer review panel within six months of receiving submission of the validation study (Submission expected in 2010).

Ocular Toxicity Testing

Goal

Two important goals in the area of ocular toxicity are to 1) identify and promote research, development, translation, and validation activities for test methods that can partially or fully replace the Draize rabbit eye test for the identification of substances that are potential ocular hazards and 2) implement procedures to avoid pain and distress where animals must still be used.

Specific Objectives

- Identify alternative test methods that can accurately predict the hazards associated with substances that cause reversible eye damage.
- Identify testing batteries that could be used to increase the accuracy for predicting all ocular hazard categories.
- Promote the inclusion of humane endpoints in current *in vivo* ocular toxicity tests.
- Promote the routine use of topical anesthetics and systemic analgesics in current *in vivo* ocular toxicity tests.

Planned Activities for Implementation

1a. Evaluate *in vitro* approaches for assessing the ocular irritation potential of antimicrobial cleaning products (both reversible and irreversible damage).

1b. Assess *in vitro* ocular toxicity test methods proposed for assessing reversible eye damage (vs. irreversible permanent eye damage).

1c. Evaluate testing strategies/batteries using multiple *in vitro* methods.

1d. Review the routine use of topical anesthetics and systemic analgesics for reducing pain and distress.

2009 Accomplishments*: An international independent scientific peer review panel, composed of 22 scientists from the U.S., Japan, Canada, the Netherlands, Belgium, and Spain met in public session on May 19-21, 2009 to evaluate alternative test methods and approaches that may further reduce and refine the use of animals for ocular safety testing. The Panel evaluated the validation status of each of the above proposed alternative test methods and approaches according to established Federal and international criteria. The Panel also commented on draft ICCVAM recommendations regarding the usefulness and limitations of each proposed test method and approach. ICCVAM will consider the Panel's report along with all public and SACATM comments and prepare final test method recommendations that it will forward to Federal agencies.*

2a. For the bovine corneal opacity and permeability (BCOP) test method, evaluate relevance and reliability using an alternative corneal holder and using alternative vehicles for the test substance diluents.

2b. For the BCOP test method, evaluate the effect of modifying various test method protocol components (e.g., duration of test substance exposure) on accuracy and/or reliability.

Timeframe: ICCVAM will propose topics for 2009/2010 NIH Small Business Innovation Research grants that could be used as a funding mechanism for interested stakeholders to

complete these studies. The precise timeline will be dependent on the level of interest and the grant application process.

3. Promote the evaluation of ocular histopathology for its potential to improve test method predictivity.

3a. Create a reference atlas for the histopathology of chemically induced ocular lesions.

3b. Develop a standardized histological scoring system and revised hazard classification decision criteria for *in vivo* and *in vitro* test methods.

3c. Encourage the submission in vitro testing results and histopathology specimens to NICEATM for characterization and to create a database for evaluation.

Timeframe: A workshop on the use of histopathology in ocular toxicity testing is proposed for Fall 2009 to bring together a working group of veterinary pathologists and toxicologists with expertise in ocular safety testing to complete these activities.

Acute Toxicity Testing

Goal

Identify mechanism-based *in vitro* test systems and earlier, more humane endpoints that can be used to further reduce, refine, and eventually replace animal use for acute systemic toxicity testing, while ensuring the protection of human and animal health.

Specific Objectives

- Identify standardized procedures for collecting mechanistic information from acute oral toxicity testing to aid in developing batteries of predictive *in vitro* test methods that can further reduce and eventually replace animals for acute toxicity testing
- Identify more objective endpoints that could be used to define evident toxicity and their use to terminate a study.
- Explore opportunities to collaborate with the European Centre for the Validation of Alternative Methods (ECVAM) on the AcuteTox Project, which has the goal of developing an *in vitro* test strategy to completely replace *in vivo* testing of chemicals for acute toxicity
- Evaluate the applicability of the Up-and-Down Procedure (UDP) and the Fixed Dose Procedure (FDP) as ways to reduce animal use for acute dermal systemic toxicity and acute inhalation toxicity, where consistent with regulatory needs

Planned Activities for Implementation

1. Organize an international workshop to identify earlier, more humane endpoints and predictive batteries of *in vitro* test methods **(Completed February 2008**)

   ***2008 Accomplishment**: The Workshop on Acute Chemical Systemic Toxicity Testing – Strategies for Developing and Advancing More Humane Endpoints and In Vitro Alternatives was convened on February 6 – 7, 2008). A report detailing the conclusions and recommendations resulting from this workshop is available on the NICEATM-ICCVAM website[4]. This report is available to ICCVAM Agencies for their consideration as possible research, development, translation, and validation activities.*

2. Work with stakeholders to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods (or batteries of tests) and earlier, more humane endpoints for acute systemic toxicity testing

3. Participate on an international Validation Management Group for a human hepatic biotransformation enzyme induction assay using HepaRG cells and cryopreserved human hepatocytes

[4] http://iccvam.niehs.nih.gov/methods/acutetox/Tox_workshop.htm

Dermal Toxicity Testing

Goal

The replacement of the rabbit skin test for corrosivity and irritation with alternative test methods that meet the requirements of U.S. regulators.

Specific Objectives

- Determine the usefulness and limitations of *in vitro* skin model systems for skin irritation testing.
- Determine how corrosive substances that have produced false negative results in *in vitro* corrosivity test methods will act in the *in vitro* dermal irritation test method protocols.

Planned Activities for Implementation

1a. Evaluate alternative dermal irritation test methods for their usefulness and limitations in U.S. regulatory testing

1b. Assist in the development of an Organisation of Economic Co-operation and Development (OECD) Test Guideline for human skin model systems for skin irritation testing.

1c. Evaluate a combination (or battery) of *in vitro* test methods for evaluating skin corrosivity and irritation

1d. Conduct a study to evaluate potential false negative corrosive chemicals in proposed *in vitro* dermal irritation assays

Timeframe: The study is scheduled for completion in Summer 2009.

Dermal Sensitization Testing

Goal

Identify and promote research, development, translation, and validation activities for test methods that can reduce, refine, or replace the use of animals to determine the potential of chemicals and products to produce allergic contact dermatitis.

Specific Objectives

- Identify adequately valid test methods that can detect potential skin sensitizers without the requirement for radioactivity.
- Identify ways to reduce the number of animals required for skin sensitization testing
- Collect and review current murine local lymph node assay (LLNA) data to determine whether the applicability domain of the LLNA can be expanded
- Explore opportunities to collaborate with ECVAM on the Sens-It-Iv project, which has the goal of developing an *in vitro* testing strategy to replace animal tests currently used for the risk assessment of potential skin or lung sensitizers.

Planned Activities for Implementation

1a. Evaluate the validation status of the LLNA as a stand-alone assay for potency determination for classification purposes

1b. Evaluate the validation status of the LLNA for testing formulations, aqueous solutions, and metals

1c. Evaluate the validation status of modified LLNA protocols that do not use radioactivity

1d. Evaluate the validation status of the reduced LLNA test method

1e. Develop test method performance standards for the LLNA that could be used to quickly and efficiently evaluate the usefulness and limitations of modified versions of the LLNA that are mechanistically and functionally similar to the traditional LLNA

2008-09Accomplishments*: An independent expert peer review panel, composed of 16 scientists from the U.S., Japan, Canada, the Netherlands, Germany, and the Czech Republic, met in public session on March 4-6, 2008 to evaluate the validation status of new versions and applications of the LLNA was convened. A report detailing the Panel's conclusions and recommendations resulting from this meeting was published in May 2008*[5]*. Based on data received since the March 2008 Panel meeting, NICEATM is re-evaluating several of these topics. The Panel was reconvened on April 28-29, 2009 to discuss updates to the original draft background review documents and draft test method recommendations. A report detailing the Panel's conclusions and recommendations resulting from this meeting was published in June 2009. ICCVAM will consider the*

[5] http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm

Panel's report along with all public and SACATM comments and prepare final test method recommendations that it will forward to Federal agencies in 2009.

2. Review and comment on computational and *in vitro* methods proposed for incorporation into testing strategies (e.g., ECVAM Sens-It-IV Project, human cell line activation test [hCLAT], the dipeptide reactivity assay, the myeloid U937 skin sensitization test [MUSST]) for identifying potential skin or lung sensitizers.

Endocrine Disruptors Testing

Goal

Identify and promote research, development, translation, and validation activities for individual *in vitro* endocrine disruptor test methods, or batteries of these methods, that can reduce the numbers of animals needed to screen for chemicals that might interfere with the endocrine systems of humans or wildlife.

Specific Objectives

- Complete a joint international study with ECVAM and the Japanese Center for the Validation of Alternative Methods (JaCVAM) to evaluate the usefulness and limitations of an *in vitro* test method to identify estrogen-like chemicals and that does not require the use of animals as donors for test components.
- Provide support in designing studies for the validation of the Certichem, Inc., MCF-7 Cell Proliferation Assay protocols for both the detection of estrogenic and anti-estrogenic activity.
- Increase involvement in OECD test guideline activities related to endocrine disruptors.

Planned Activities for Implementation

1a. Further standardize and optimize the agonist and antagonist protocols for the LUMI-CELL® ER assay and test the 78 ICCVAM recommended substances for the validation of *in vitro* ER TA test methods, in three laboratories (one in Europe, one in Japan, and one in the United States), to evaluate test method reliability (intralaboratory repeatability, intra- and inter-laboratory reproducibility) and comparative performance against the NICEATM meta-data for ER active compounds.

1b. Use the results from the testing of the 78 ICCVAM recommended substances to develop a high quality *in vitro* ER TA database and performance standards that can be used to characterize the extent to which other *in vitro* endocrine disruptor test methods (or test method batteries) might be used to further reduce the requirements for animal use in the screening of potential endocrine disruptors.

Timeframe: The validation study is scheduled for completion in Fall 2009, with an ICCVAM expert peer review panel meeting scheduled for Spring 2010.

2a. Provide comments on study design for the validation of the Certichem, Inc., MCF-7 Cell Proliferation Assay protocols for both the detection of estrogenic and anti-estrogenic activity.

2b. Provide CertiChem, Inc., with coded samples of each of the compounds in the list of 53 ICCVAM recommended reference substances considered as the minimum for the validation of ER TA test methods.

Challenge #2: Incorporating New Science and Technology

The second challenge is to identify and promote research incorporating new technologies that can be expected to support the future development of new test methods and approaches to reduce or eliminate the need for animals. While many of these approaches will require several years to develop and validate, some may be ready for use more quickly. To maximize the efficiency of this process, NICEATM and ICCVAM are working with Federal agencies and other stakeholders to link research and development activities to the standardization and validation of alternative test methods that may be used in regulatory testing.

ICCVAM Agencies have been surveyed for ongoing and planned research, development, translation, and validation activities relevant to test methods that reduce, refine, and replace the use of animals. A table of these activities is included as **Appendix A**. As part of this implementation plan, as appropriate, the role of ICCVAM working groups will be expanded to charge members with interacting with test method developers who would benefit from such consultation.

An ICCVAM Research and Development Working Group (RDWG) has been established to assist with implementation of activities relevant to incorporating new science and technology as outlined in the Five-Year Plan. The RDWG is specifically charged with aiding NICEATM and ICCVAM in identifying and promoting research incorporating new technologies that can be expected to support the future development of new test methods and approaches that will reduce, refine, and replace animal use in toxicity testing. NICEATM and ICCVAM seek to link research and development activities to the standardization and validation of alternative test methods that may be used in regulatory testing. Consultation and cooperation with NICEATM and appropriate ICCVAM Test Method Working Groups during test method development and validation is expected to maximize the value of such test methods and approaches to regulatory agencies. The RDWG will be asked to help identify test methods in the development phase for referral to appropriate Test Method Working Groups.

Nanomaterials Testing

Goal

Identify and promote research, development, translation, and validation activities for test methods that can reduce, refine, or replace the use of animals in regulatory safety testing for nanomaterials.

Specific Objectives

- Work with stakeholders to identify test methods that are considered to be most appropriate for nanomaterials
- Foster the development and evaluation of alternative methods for nanomaterials testing.
- Define the current and planned activities within ICCVAM agencies (or that are supported by ICCVAM agencies) that are relevant to nanomaterials testing and the use of alternative test methods

Planned Activities for Implementation

1. Assess the state of the science to determine if developing a scientific workshop to evaluate possible alternatives is warranted. Goals of such a workshop would be to:

    - Identify and prioritize future research initiatives necessary to advance development and validation of *in vitro* methods for nanomaterials testing.
    - Discuss how to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of *in vitro* test methods for nanomaterials testing.

Timeframe: Contingent on outcome of assessment. Currently coordinating with U.S. interagency and OECD working groups conducting similar activities.

2. Develop a one-day symposium to define the current planned activities with ICCVAM agencies that are relevant to nanomaterials testing and the use of alternative methods.

    - ICCVAM agencies would be asked to identify current or new members with expertise specific to nanomaterials to each working group that could be involved in developing the proposed workshop.

3. Become engaged in OECD activities relevant to alternative test methods intended for safety testing of nanomaterials

    - An ICCVAM and/or NICEATM representative will be proposed as a member of the OECD steering group that is reviewing existing alternative methods and making recommendations on test methods considered to be most appropriate for nanomaterials.

High Throughput Screening

Goal

Identify batteries of rapid biochemical- or cell-based high throughput screening (HTS) assays that may reduce or replace the use of animals in toxicological tests.

Specific Objectives

- Facilitate the review of the usefulness and limitations of defined HTS approaches, and also assist in the identification of assays and endpoints that are relevant for alternative test methods that have already been adopted.

Planned Activities for Implementation

- Monitor progress in collaborations between research institutes within three ICCVAM Agencies (NIEHS [National Toxicology Program], EPA [National Center for Computational Toxicology], and NIH [Center for Chemical Genomics]) that will test a large number of compounds (~ 10,000) broadly characterizing and defining the chemical-biological space occupied by chemicals of toxicological concern. This collaboration will establish a spectrum of secondary and tertiary-screening assays to further define and

513 characterize activities identified in initial high throughput screens. The goals
514 of this interagency program are to:

515 – Prioritize substances for further in-depth toxicological evaluation (to
516 judiciously allocate efforts and resources to maximize public health
517 impact).

518 – Identify mechanisms of action for further investigation (e.g., disease-
519 associated pathways).

520 – Develop predictive models for *in vivo* biological response (predictive
521 toxicology).

522 • Nominate substances identified by NICEATM/ICCVAM as reference
523 compounds for the development of alternative test methods as well as other
524 compounds that have been tested in various alternative test methods, in the
525 standard *in vivo* toxicity tests, or in humans.

Challenge #3: Fostering Acceptance and Appropriate Use of Alternative Test Methods

Once regulatory authorities have accepted an alternative test method, ICCVAM will work to promote its use by broadly communicating the outcomes of ICCVAM review activities and/or workshops via the *Federal Register*, at national or international scientific meetings, via peer reviewed journal publications, and at training courses. Emphasis will also be placed on making the scientific community, including Institutional Animal Care and Use Committees (IACUCs), aware of new alternatives that are available for consideration in complying with the PHS Policy and Animal Welfare Act provisions, which state that such methods must be considered prior to testing in animals, where applicable.

ICCVAM will collaborate with government and non-governmental organizations, where appropriate, to co-sponsor workshops. The objectives of these workshops will be to evaluate the state-of-the-science related to the development and validation of alternative toxicological test methods, and to identify high priority research, development, translation, and validation activities necessary to advance and characterize the usefulness of such methods. The results of these workshops will be broadly communicated to individuals and organizations that conduct such activities.

NICEATM-ICCVAM Website

Goal

- Provide user-friendly access to the latest information on validation processes and the most up-to-date status of the alternative test methods previously reviewed and those currently under review.
- Provide access to publicly available reference test method databases for use in the development and validation of alternative test methods.
- Promote active communication and outreach efforts with both government and non-government stakeholders.

Specific Objectives

- Use a combination of e-mail and website announcements to inform the public of the availability of newly published Federal Register notices, NICEATM documents, journal articles, and upcoming events

Planned Activities for Implementation

1a. Create agency websites dedicated to their specific activities associated with alternative test methods research, development, translation, and validation. NICEATM/ICCVAM will in turn provide a link on their website to these member agency websites.

1b. One or more lists of frequently asked questions (FAQs) will be developed to provide quick reference guides to broad issues related to the ICCVAM test method evaluation process, as well as more specific issues relevant to individual toxicity testing areas.

- The content for each website dedicated to alternative test method activities can include the following information:
  - Currently Available Alternative Methods
  - Validation and Acceptance Process for Alternative Methods
  - Regulations and Applicability to Alternative Methods
  - Alternative Methods Research, Development, and Translation Activities
  - Frequently Asked Questions
  - Resources

2. Create a web-based database of all test methods that have been reviewed or that are currently undergoing review.

2009 Accomplishments: The NICEATM-ICCVAM website has been updated to include a web-based database of all test methods that have been reviewed or that are currently under review, including their development, validation, evaluation, and regulatory acceptance status.

Challenge #4: Developing Partnerships and Strengthening Interactions with ICCVAM Stakeholders

ICCVAM will also foster international collaboration by including experts from the international scientific community on workshops that review the state of the science for particular test method areas. Where appropriate, NICEATM and ICCVAM will also invite representatives from international organizations such as OECD and from OECD member countries to attend and participate in relevant NICEATM and ICCVAM-sponsored workshops, peer reviews, and other scientific activities. Similarly, to further ensure the development of scientifically valid international test guidelines, NICEATM and ICCVAM will seek to increase participation of its scientists in U.S. delegations to OECD test guideline meetings, expert consultations, and workshops.

Goal

- Develop partnerships and strengthen interactions with ICCVAM stakeholders to promote research, development, translation, and validation activities for alternative test methods.

Specific Objectives

- Be more proactive in identifying research needs and promising methods that should be priorities for further development, translation, validation, or ICCVAM evaluation.
- Foster interagency collaboration among Federal research and regulatory agencies, including opportunities for test method validation activities.
- Strengthen international relationships with appropriate organizations to foster the validation and evaluation of alternative test methods.
- Foster international collaboration by including experts from the international scientific community on expert panels and workshops.

Planned Activities for Implementation

1. Collaborate with government and non-governmental organizations, where appropriate, to co-sponsor workshops. The objectives of these workshops will be to evaluate the state-of-the-science related to the development and validation of alternative toxicological test methods, and to identify high priority research, development, translation, and validation activities necessary to advance and characterize the usefulness of such methods. The results of these workshops will be broadly communicated to individuals and organizations that conduct such activities.
2. Facilitate the international adoption of valid alternative test methods by providing standardized protocols that can be considered for adoption by international organizations (for example, the International Standards Organization [ISO], OECD, etc.). As appropriate, NICEATM and ICCVAM will provide comprehensive test method background review documents and the results of independent scientific peer reviews to facilitate the approval of these test methods by the international community.

2008-2009 Accomplishments:

- *ICCVAM, in conjunction with stakeholders in the United States, the European Union (EU), and Japan, drafted an OECD Test Guidelines (TG) for the Isolated Chicken Eye (ICE) and the BCOP test methods. The TGs were developed following an international peer review evaluation with contributions from ECVAM and JaCVAM. The draft TGs were recently accepted by the OECD Working Group of National Coordinators. Once formally adopted by the OECD Council, TG 437 will be accepted by all 30 OECD member countries in accordance with OECD Mutual Acceptance of Data. The use of these TGs will reduce the use of rabbits for eye safety testing and eliminate such testing in animals of most substances likely to cause severe pain and discomfort.*
- *NICEATM, in conjunction with the ICCVAM Acute Toxicity Working Group, drafted an OECD Guidance Document entitled, In Vitro Neutral Red Uptake (NRU) Cytotoxicity Tests for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests. The standardized protocols on which this draft GD is based were developed during a NICEATM/ICCVAM/ECVAM sponsored international validation study.*
- *NICEATM and the ICCVAM Genetic Toxicity Working Group (GTWG) are involved in development of a draft OECD TG for the in vitro mammalian cell micronucleus assay and have provided comments on a study to determine the most appropriate measure of cytotoxicity for inclusion in the TG.*

3. Work with other national and international validation organizations (for example, ECVAM and JaCVAM) to promote ICCVAM's validation and acceptance criteria, which have been substantially incorporated into OECD Guidance Document 34, and to consider other issues related to validation as they occur.

***2008-2009 Accomplishments**:*

- *Signed a Memorandum of Cooperation (MoC) that seeks to establish an International Cooperation on Alternative Test Methods (ICATM) in order to expand and strengthen cooperation, collaboration, and communications among national validation organizations on the scientific validation and evaluation of new alternative testing methods proposed for regulatory health and safety assessments. The MoC has been adopted by the current ICATM Validation Organizations (i.e., ECVAM, ICCVAM, JaCVAM, and Health Canada).*
- *NICEATM and ICCVAM representatives are serving on the Validation Management Group for a prospective validation of Reconstructed Human Tissue models for identification of mild to moderate irritants and substances not labeled as ocular irritants.*
- *NICEATM and ICCVAM representatives are participating with ECVAM and JaCVAM in the Validation Management Group for in vitro approaches to skin sensitization testing.*

- *NICEATM and ICCVAM representatives are involved in development of the validation study plan for the in vivo rodent and in vitro alkaline comet assay for detection of genotoxic carcinogens, the proposed protocol, and proposed list of reference substances, and ICCVAM has representatives on the Validation Study Management Team*
- *NICEATM and ICCVAM representatives provided comments to JaCVAM on their validation study plan and protocol for their validation study of the cell transformation assay as well as serving as providing liaison members to the Validation Study Management Team*
- *NICEATM and ICCVAM participants are providing input and guidance to an ECVAM Validation Study of a human hepatic biotransformation enzyme induction assay using HepaRG cells and cryopreserved human hepatocytes*

4. Participate in the development of performance standards for international test guidelines.

***2008-2009 Accomplishments**:*

- *ICCVAM and NICEATM, in conjunction with ECVAM and JaCVAM developed internationally harmonized performance standards for the murine local lymph node assay (LLNA) and submitted Special Project Submission Forms (SPSFs) to OECD for updating OECD TG 429 (the LLNA) with these performance standards*
- *ICCVAM and NICEATM also submitted SPSFs to OECD for updating OECD TG 430 (rat skin TER), and OECD TG 431 (Human skin model systems) with performance standards previously developed by ICCVAM*

5. To further ensure the development of scientifically valid international test guidelines, NICEATM and ICCVAM will seek to increase participation of its scientists in U.S. delegations to OECD test guideline meetings, expert consultations, and workshops.

***2008-2009 Accomplishments**:*

- *NICEATM and ICCVAM representatives participated in an OECD Expert Consultation during the development of the aforementioned TGs for ICE and BCOP.*
- *NICEATM and ICCVAM representatives are participating as members of an OECD Expert Working Group on a draft TG for human skin model systems and will host an Expert Consultation meeting in June 2009*
- *NICEATM and ICCVAM provided nominations of independent experts to serve on an ESAC peer review panel for the cell transformation assay*
- *NICEATM and ICCVAM provided nominations of independent experts to serve on an ESAC peer review panel of four cell function-based in vitro methods (fluorescein leakage, neutral red release, cytosensor microphysiometer and red blood cell haemolysis test methods) for*

identification of mild to moderate irritants and substances not labeled as ocular irritants.

6. Invite representatives from international organizations such as OECD and from OECD member countries to attend and participate in relevant NICEATM and ICCVAM-sponsored workshops, peer reviews, and other scientific activities.

Recent Accomplishments:

- *In 2008, ICCVAM recommended five in vitro pyrogenicity test methods measuring cytokine release from human cells as replacements for the rabbit test, subject to product-specific validation, to detect endotoxin contamination in parenteral drugs. These recommendations were finalized following consideration of conclusions and recommendations from an independent peer review panel that included members from five different countries.*
- *In 2008, ICCVAM completed reviews of the reduced LLNA rLLNA and LLNA performance standards and subsequently forwarded recommendations to Federal agencies. These recommendations were finalized following consideration of conclusions and recommendations from an independent peer review panel that included members from eight different countries. This Panel recently reviewed additional data relevant to the applicability domain of the LLNA and three non-radiolabeled LLNA methods.*
- *An independent peer review panel that includes members from six different countries is reviewing the validation status of several methods relevant to ocular safety testing. The public meeting was held on May 19-21, 2009.*

7. Engage interested stakeholders in assessing how to efficiently meet Federal peer review requirements, and seek input on ways to streamline processes that will not compromise transparency, scientific rigor, or the opportunity for stakeholder participation.

Appendix A Ongoing and Planned Research, Development, Translation, and Validation Activities in ICCVAM Member Agencies Relevant to Test Methods That Reduce, Refine, And Replace The Use Of Animals[1]

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Targeted testing areas (High throughput screening and computer modeling)	EPA/ORD /NHEERL	Ongoing	ToxCast	Res Devel	EPA/ORD/NCCT will continue to develop a toolbox (ToxCast) for prioritizing chemicals for toxicology evaluation, providing computational models that will define bioactivity profiles of chemicals using a variety of high throughput high content screening assays. The ToxCast project is starting with a proof of concept effort that is collecting data on 300 pesticides. Algorithms will be developed to match the bioactivity data to known toxicological phenotypes. In the next phase of the project, the chemical space and bioactivity profile descriptions will be expanded with a larger set of chemicals as a validation of the proof of concept. If the preliminary phases are successful, the project will proceed to an implementation phase where bioactivity profiles of chemicals in need of toxicological evaluation will be obtained and recommendations for testing priorities will be provided as the final outcome.	All 3	
Aquatic toxicity	EPA/ORD /NHEERL	Ongoing and future	Development of fish and amphibian assays	Res Devel	EPA/ORD/NHEERL will continue to develop assays to evaluate various toxicity endpoints in fish and amphibians.	Replace	
Aquatic toxicity	EPA/ORD /NHEERL	Ongoing and future	Development of amphibian metamorphosis assay	Res Devel	EPA/ORD/NHEERL will continue to participate in the validation of an assay to evaluate amphibian metamorphosis.	Replace	
Targeted testing areas	EPA/ORD /NHEERL	Ongoing and future	Development of mammalian assays	Res Devel	EPA/ORD/NHEERL will continue to develop assays to evaluate various human health toxicity endpoints in rodents.	Refine	
Targeted Testing	ATSDR	Ongoing	Development of methods for mixtures toxicity evaluation	Res Devel	Through cooperative agreements with universities, the Agency, conducts hypothesis driven research to evaluate toxicity of chemical contaminants and their mixtures	All 3	Environ-mental Tox Pharma Vols 16, 18

[1] NOTE: This table is a compilation of responses received in response to the original request during the development of the NICEATM-ICCVAM Five-Year Plan. A memo requesting that ICCVAM Principal Agency Representatives have their respective sections updated will be forthcoming.

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Targeted Testing Areas	NIEHS	Future Possible Activities	1. Targeted Research Grants 2. SBIRs (Devel And prevalidaton) 3. Validation Contracts 4. NICEATM validation studies	Res Devel Trans Valid	NICEATM works with ICCVAM to organize Workshops, Scientific Symposia and Expert Panels to identify high priority research, development, translation, and validation activities considered necessary to advance alternative test methods for specific toxicity endpoints. These are potential mechanisms available to carry out high priority activities.	All 3	Reports of Workshops, Symposia, and Expert Panels
Acute Systemic Toxicity	ATSDR	Ongoing	Predictive Toxicity Methods	Res Devel Valid	Uses available computational tools and approaches to evaluate toxicity of chemicals for screening and prioritizing chemicals for further research and analysis.	All 3	
Acute Systemic Toxicity	DOI	Near-future	Revised test protocol for evaluation of candidate nontoxic shot	Devel	A three-tiered testing strategy was previously developed and approved that includes toxicological protocols for testing candidate nontoxic shot in animals. The proposed activity is modifying the protocol to include solubility testing in tier one of the testing strategy.	Reduce	
Acute Systemic Toxicity	FDA	Ongoing	Special adaptation of OECD Test Guideline 425 (TG 425 - Up and Down Procedure) for selection of dose range for toxicity study	Res Devel	Use of a modified OECD TG 425 to select appropriate doses for a subsequent larger second study of the acute oral toxicity. Three modifications to TG 425 were used: 1) A different formula for increasing and decreasing the dose was used. 2) Treated rat were observed for more than 48 hrs. 3) The primary endpoint was not mortality.	Reduce	
Acute Systemic Toxicity	NIEHS	Ongoing	Monitor and collaborate with ECVAM on the A-Cute-Tox Project	Res Devel Trans Valid	NICEATM and the ICCVAM/ATWG will monitor progress and provide input for ECVAM's A-Cute-Tox Project to develop *in vitro* tests and other methods necessary to achieve accurate acute oral hazard classification in order to further reduce and potentially replace animals for this purpose. The ECVAM project implements recommendations from the 2000 ICCVAM Workshop on this topic.	Reduce, Replace	Key references: Acute Toxicity TMER, BRD, Workshop Report, Guidance Document

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Acute Systemic Toxicity	NIEHS	Ongoing	Expand LD50 database	Res Devel Trans Valid	NICEATM will continue to collect and make available high quality existing in *vivo* rat acute oral toxicity test data that can be used as reference data to develop and validate in vitro methods. NICEATM will identify appropriate LD_{50} values and calculate reference values	All 3	
Acute Systemic Toxicity	NIEHS	Near-future	Mechanisms of Acute Systemic Toxicity and Lethality Workshop	Res	NICEATM and ICCVAM will organize an international workshop to identify and standardize procedures for collecting information pertinent to an understanding of mechanisms of lethality and acute systemic toxicity in rats that will facilitate development of in vitro test systems that can predict these toxic effects.	All 3	
Acute Systemic Toxicity	NIEHS	Near-future	QSAR Evaluation	Res Devel Trans Valid	NICEATM will use available quantitative structure-activity relationship [QSAR] software and compare with the reference LD50 values determined for the validation study reference substances to estimate starting doses for acute oral toxicity testing.	All 3	
Acute Systemic Toxicity	NIEHS	Future	*In Vitro* Cytotoxicity of Mixtures	Valid	Determine the usefulness of the 3T3 NRU test method for reducing and refining the use of animals for the acute oral systemic toxicity testing of chemical mixtures 1. Collect acute historical oral LD50 values for mixtures from standardized acute oral toxicity test methods with rats (provided by regulatory agencies and/or chemical manufacturers). 2. Prospectively test mixtures using *in vitro* 3T3 NRU as they undergo mandatory *in vivo* safety testing by industry, where in vivo data will be made publicly available	All 3	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Acute Systemic Toxicity	NIMH	Ongoing	Toxicological Evaluation of Novel Ligands Program	Trans Valid	This contract provides support to investigators in the preclinical development of novel imaging agents. As part of this program, the contractor conducts preliminary dose-range finding toxicity studies by a variety of methods, which are selected on a case-by-case basis based on chemical structure, availability of previous data (e.g., animal efficacy data), and other information. Wherever possible, the contractor employs the ICCVAM-approved methods (modified up-and-down procedure, in vitro predictive models) for accurate prediction of dose levels before moving into full toxicology assays required by the FDA.	Reduce Refine	
Biologics/ Vaccines	FDA	Near-future	Development of surrogate tools to evaluate pre (non)-clinical efficacy of S-protein based SARS-CoV vaccines in cell culture as an alternative to the animal challenge studies	Res Devel	Development of surrogate tools that can be used in cell culture as an alternative to the use of animals in pre-clinical challenge/efficacy evaluation of SARS-CoV S protein-based vaccines	Reduce, Replace	
Biologics/ Vaccines	FDA	Ongoing	In vitro assays of vaccine efficacy and correlates of protection for vaccines for intracellular pathogens	Res	1) Development an *in vitro* tissue culture assay that measures the ability of T cells from mice sublethally infected with *Mycobacterium tuberculosis* (*M. tb*.), and thus immune, to reduce intracellular bacterial growth when co-cultured with *M. tb*.-infected macrophages. 2) Development of an *in vitro* tissue culture assay that measures the ability of T cells from mice sublethally infected with *Francisella tularensi*s LVS (LVS) co-cultured with LVS-infected macrophages to reduce intracellular bacterial growth.	Reduce, Refine	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Biologics/ Vaccines	FDA	Near-future	Refinement of Diphtheria vaccine potency testing: Elimination of lethal challenge models and movement toward international harmonization	Valid	1) Evaluate whether the inclusion of a control vaccine in the US test significantly improves assay precision 2) Investigate the minimal dose of D toxoid in International Units that satisfies the US requirements, making both methods comparable. 3) An in vitro assay, currently under study, will be used instead of the lethal challenge to measure the neutralizing antibody response in guinea pigs.	Reduce, Replace	
Biologics/ Vaccines	FDA	Near-future	Evaluation of vaccinia replication and dissemination in vivo: New endpoints to eliminate death and suffering of animals for evaluation of therapeutic agents, passive immunity and prophylactic vaccines	Res Devel	Development of a method that uses a recombinant vaccinia, expressing the reporter genes B-galactosidase (B -Gal) or luciferase, to follow vaccinia dissemination to internal organs in normal animals and in several knockout mouse strains.	All 3	
Biologics/ Vaccines	FDA	Near-future	Development of an alternative pre-clinical assay to test immunogenicity of vaccines using human antigen-presenting cells (APC) in-vitro	Res Devel Trans Valid	This surrogate assay is based on the requirement for the presence of the relevant vaccine-derived peptides on the surface of APC for the induction of potent cellular response. Using HPLC analysis, vaccine-derived peptides complexed with human MHC class I/II molecules will be identified and quantified on the surface of human APCs.	Reduce, Replace	
Biologics/ Vaccines	FDA	Near-future	Development of in vitro quantitative assays to be used as vaccine potency release criteria to replace in vivo animal immunogenicity assays	Devel	Development of a set of in vitro quantitative assays to measure the levels of transcription and translation that should be sensitive to loss of potency and be predictive of in vivo immunogenicity.	Replace	
Biologics/ Vaccines	NIEHS	Ongoing	Botulinum Toxin Workshop Report	Res	NICEATM will publish a workshop report detailing the discussions and output from the meeting	All 3	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Biologics/ Vaccines	USDA	Ongoing	Development of Quantitative Assay and Physiochemical Correlates of Biological Activity for *Clostridium haemolyti*cum beta toxin (phospholipase C)	Res	Identification of a protective immunogen and development of an in vitro potency test for *C. haemolyticum* bacterin-toxoid.	Replace	
Biologics/ Vaccines	USDA	Ongoing	Development of in vitro assays for measuring the relative potency of leptospiral bacterins containing serovars pmona, canicola, grippotyphosa and icterohaemorrhagiea	Valid	Validation of the leptospira bacterin ELISA potency test.	Replace	
Biologics/ Vaccines	USDA	Near-future	Development of an in vitro rabies potency test	Res	Development of an in vitro assay for rabies vaccines, potentially in conjunction with FDA and CDC.	Replace	
Chronic Toxicity/ Carcino-genicity	FDA	Near-future	Analysis of p53 Codon 270 CGT to TGT Mutation in Simulated Solar Light-induced Skin Tumors and Exposed Mouse Skin	Res	Development of a method to detect a mutation in mice at p53 codon 270 (CGT->TGT), and measure: 1) the frequency of detection and levels of this mutation in mouse skin tumors 2) the frequency of this mutation in skin tissue from tumor-bearing animals 3) the frequency of this mutation in skin exposed to decreasing levels of SSL.	Reduce	
Chronic Toxicity/ Carcino-genicity	FDA	Near-future	Measurement of Cancer-Associated Gene Mutation in Colon Tumor and non-Tumor Tissue	Res	Determination of k-ras codon 12 GGT to GAT and GGT to GTT mutant frequencies in: 1) colonic ACF, adenomas, and carcinomas 2) tumor margin samples and tumor-distant, normal-appearing colonic epithelium from colon cancer patients 3) autopsy samples of colonic epithelium from colon-disease-free individuals.	Reduce	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Dermal Toxicity	NIEHS	Ongoing	Evaluation of EpiDerm™ and EPISKIN™ Dermal Irritation Assays for Classifying Dermal Irritants	Valid	NICEATM will support the ICCVAM Evaluation of EpiDerm™ and EPISKIN™ in vitro dermal irritation assays for predicting US (i.e., EPA and FHSA) and GHS hazard classifications for dermal irritants, and the independent scientific peer review, and development of ICCVAM recommendations for agencies	All 3	
Dermal Toxicity	NIEHS	Near-future	Evaluation of EpiDerm™ and EPISKIN™ Dermal Irritation Assays for Identifying Corrosive Compounds Not Detected in *In Vitro* Corrosivity Assays	Res Devel Trans Valid	ICCVAM has concluded that an evaluation is needed of the EpiDerm™ and EPISKIN™ dermal irritation assays for their utility in identifying corrosive substances that are false negatives in *in vitro* corrosivity tests, a pre-requisite for consideration of these methods as a way to make dermal assessments without the use of any animals. This was endorsed by ICCVAM as a high priority activity.	All 3	
Dermal Toxicity	NIEHS	Near-future	PPDC Antimicrobial Project	Valid	NICEATM will support the ICCVAM evaluation of non-animal methods and approaches for determining the skin irritation potential of antimicrobial cleaning product formulations, including an independent scientific peer review meeting.	All 3	
Endocrine Active Substances 'endocrine disruptors'	NIEHS	Ongoing	International Validation Study of the LUMI-Cell ER TA Assay	Valid	Joint Validation Study with ECVAM and JaCVAM to validate the LUMI-Cell ER TA assay for detecting agonists and antagonists		NICEATM XDS Standardization Report
Endocrine Active Substances 'endocrine disruptors'	NIEHS	Near-future	International Validation of the CERI ER TA Assay for Detecting Antagonists	Valid	JaCVAM requested that NICEATM consider participating in an international validation study of the CERI ER TA Antagonist assay, after OECD has finished peer review of the agonist assay.		
Endocrine Active Substances 'endocrine disruptors'	NIEHS	Near-future	Validation of the CertiChem MCF-7 ER TA Assay	Valid	ICCVAM has concluded that the CertiChem cell-based estrogen receptor transcriptional activation test method, which measures cell proliferation in MCF-7 cells, is suitable for validation and endorsed such a study as a high priority activity.		

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Genetic Toxicity	FDA	Ongoing	An Efficient Regulatory Method for Evaluating Chromosomal Damage: Analysis of Micronucleus in Different Rat Strains by Flow Cytometry	Res	Provide the information necessary to establish a new standard for pre-market evaluation of genotoxic potential by: 1) Establishing the validity of the flow cytometric scoring of micronuclei in Sprague-Dawley and Fischer 344 rats; 2) Determining the kinetics of the appearance and elimination of micronucleated cells in both strains; and 3) Determining whether the frequency of micronuclei in the young circulating reticulocytes accurately reflects the frequency in the primary bone marrow cell population from which they are derived.	Reduce	
Genetic Toxicity	FDA	Near-future	Genotoxicity, Mutagenicity, and Exposure Biomarkers of Acrylamide and Its Metabolite, Glycidamide, in Rodents: HPRT and TK+/- Mutagenesis Assay	Res	Comparison of the extent of DNA adduct formation, induction of micronuclei, and mutagenicity of acrylamide and its metabolite glycidamide in B6C3F1/Tk+/- mice treated neonatally.	Reduce	
Genetic Toxicity	FDA	Near-future	DNA Adducts of Tamoxifen	Res	This project will characterize DNA adducts from suspected tamoxifen metabolites, and develop methods for their detection and quantitation	Reduce	
Genetic Toxicity	FDA	Near-future	Evaluation of the Types of Genetic Events Detected by the Mouse Lymphoma Assay (MLA) and the Role of the Assay in Mechanistically-Based Risk Assessment	Res	1) Determine if the L5178Y/TK+/- Mouse Lymphoma Assay adequately detects both aneuploidy and mitotic recombination 2) Determine if the L5178Y mouse lymphoma cells have active recombinase functions which lead to a large proportion of mutants that result from recombinase-mediated rearrangements 3) Determine what is/are the fundamental genetic mechanism(s) causing the small and large colony thymidine kinase mutant phenotypes.	Reduce, Replace	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Genetic Toxicity	FDA	Near-future	Transgenic Mouse Model for Detecting In Vivo Mutation Using a Green Fluorescent Protein Reporter	Res	Development of two lines of transgenic mice expressing the tetracycline-repressor protein for use in: 1) Investigations of the efficiency of in vivo repression of green fluorescent protein (GFP) in various tissues of different lines of the double-transgenic mice 2) Determining the frequency of spontaneous and y-ray-induced TetR mutation in lymphocytes of double-transgenic mice using flow cytometry.	Reduce	
Genetic Toxicity	FDA	Near-future	Phosphatidylinositol glycan - Complementation Group A (PIG-A) Mutagenesis: Development of Methods for the Identification and Molecular Characterization of Mutations in the PIG-A Gene in Human Lymphoblastoid Cells and C57Bl/6 Mice	Res	1) Development of flow cytometric methods for the detection of cells with mutations in the PIGA gene using wild-type and mutant human lymphoblastoid cells, TK6 and WTK1, as a model 2) To develop flow cytometric methods for the detection of hematopoietic cells with mutations in the Pig-A gene in C57Bl/6 mice.	Reduce	
Genetic Toxicity	NIEHS	Ongoing	International Validation of the *In Vivo* and *In Vitro* Comet Assays	Valid	Participate on Study Management Team with ECVAM in JaCVAM-sponsored international validation of (1) the *in vivo* Comet assay as a replacement for the currently accepted *in vivo* rat hepatocyte UDS assay, and (2) the *in vitro* Comet assay as a potential screening assay/replacement for the *in vivo* Comet assay	All 3	
Genetic Toxicity	NIEHS	Ongoing	*In Vitro* Cell Transformation Assays	Valid	Support ICCVAM commenting on the OECD draft report on the validation status of *in vitro* cell transformation assays as a screening test/replacement for cancer bioassays	All 3	
Genetic Toxicity	NIEHS	Ongoing	*In Vitro* Micronucleus Test	Valid	Support ICCVAM commenting on the validation status of the *in vitro* micronucleus test as an OECD test guideline, as an alternative to the *in vitro* chromosomal aberration test	Replace	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Immuno-toxicity	FDA	Near-future	New Vaccine Adjuvants: Development Of Detector Cell Lines For Rapid Evaluation Of Activity And Safety	Res Devel	The goal of this proposal is to develop several types of human detector cell lines. Such detector cells will help to identify the mode of action of new adjuvants and to select against compounds that are inducing very high levels of pro-inflammatory cytokines and chemokines, which are likely to result in unacceptable toxicities in vaccine recipients.		
Immuno-toxicity	NIEHS	Ongoing	Validation Status of the LLNA: • Cut-down test • Non-radioactive tests • Potency Categorization • Performance standards	Valid	Support ICCVAM Evaluation of the validation status of the murine local lymph node assay for: • Using a single dose level (cut-down test) • Using non-radioactive versions • Making potency determinations • Developing performance standards	All 3	ICCVAM LLNA report
Neurotox-icity	FDA	Ongoing	Mumps vaccine preclinical neurotoxicity testing: moving from monkeys to rats to cell culture	Res Devel	Characterize the relative human neurotoxicity potential of several mumps virus strains and then to infect human and rat neuronal and non-neuronal cell lines with these viruses. By studying the kinetics of virus growth and spread in these cell lines and associated cellular responses, it is anticipated that a number of in vitro biomarkers of neurotoxicity can be identified.	Reduce, Replace	
Neurotox-icity	FDA	Near-future	Drosophila and Xenopus models of HSV neurovirulence, latency, and reactivation	Res Devel	Determine whether Xenopus larvae and/or Drosphilia cap cells can be used as a model for testing of Herpes simplex viruses.	Reduce, Refine	
Neurotox-icity	FDA	Near-future	Neurotoxicity Assessment of Manganese Nanoparticles in PC 12 Cells and in Mice	Res	Evaluation of the neurotoxicity of different sizes of manganese nanoparticles using PC-12 cultured cells and the identification of biomarkers of neurotoxicity in the mouse brain..	Reduce	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Neurotox-icity	FDA	Near-future	The Role of Mitochondrial Energy Disruption in the Mechanism of Neurotoxicity: Neurophysiological, Neurochemical, and cDNA Microarray Approaches	Res	Identification of biomarkers of neurotoxicity associated with exposure to 3-Nitroproprionic acid (3-NPA) and L-carnitine	Reduce	
Neurotox-icity	FDA	Near-future	NMDA Antagonist/GABA Agonist-induced Cell Death in the Developing Rat Brain	Res	Identification of biomarkers of neurotoxicity associated with exposure (one-time or prolonged) to NMDA antagonist/GABA agonists.	Reduce	
Neurotox-icity	NIH - NINDS	Near-future	Engineering form and function in neuronal networks	Res	Development of the ability to design and implement robust in vitro neural circuits on biochips that allows activity monitoring. Such an approach would allow neural circuits to be used as a test bed for neuroactive drug and toxin testing	Reduce	
Neurotox-icity	NIH - NINDS	Ongoing	Neural cell based assays derived from human ES cells	Res	Development of kits containing the reagents to propagate and reliably differentiate an improved embryonic stem cell line (WA09) primary cultures of neurons and glial cells, key cells in nervous system. The expected outcome is that researchers will have increased access to human cells of the nervous system for pharmacological and toxicological studies.	Reduce, Replace	
Ocular Toxicity	NIEHS	Ongoing	Topical Anesthetics/ Systemic Analgesics and the Draize Eye Test	Valid	Develop Background Review Document to support ICCVAM recommendations on potential routine use of topical anesthetics and/or systemic analgesics in the rabbit eye test to eliminate or reduce pain and distress associated with this procedure	Reduce	Symposium Report
Ocular Toxicity	NIEHS	Ongoing	Detection of Mild/ Moderate Eye Irritants Using BCOP, IRE, ICE, or HET-CAM	Valid	Support the ICCVAM evaluation of 4 *in vitro* ocular toxicity test methods for identifying mild/moderate eye irritants by preparing comprehensive background review documents.	Reduce	ICCVAM Ocular TMER and 4 BRDs

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Ocular Toxicity	NIEHS	Ongoing	ALTTOX		Development of a publicly available database of existing *in vivo* rabbit eye test data		
Ocular Toxicity	NIEHS	Ongoing	Development of a Histopathology Atlas and Associated Decision Criteria for the *In Vivo* Rabbit Eye Test and for *In Vitro* Tests that use Intact Eyes or Corneas	Devel Trans	NICEATM (in partnership with ECVAM and JaCVAM) will create an international working group to facilitate the collection of reference micrographs of chemically induced ocular lesions in excised corneas and enucleated eyes used in an *in vitro* ocular toxicity test method (rabbit, chicken, pig, bovine) and from eyes of rabbits used in *in vivo* tests. NICEATM will use the detailed reference atlas of chemically induced ocular lesions to create a standardized scoring system for the evaluation of these lesions. Decision criteria for the BCOP, ICE, and IRE test methods will be revised to utilize histological endpoints as a component for hazard classification.	All 3	
Ocular Toxicity	NIEHS	Near-future	PPDC Antimicrobial Project	Valid	Review of non-animal methods and approaches for determining the eye irritation potential of antimicrobial cleaning product formulations	All 3	
Ocular Toxicity	NIEHS	Near-future	Validation of the BCOP Assay using a Different Corneal Holder and Test Compound Diluent	Valid	NICEATM and ICCVAM, working in partnership with interested stakeholders, will manage a study, using ICCVAM recommended reference substances, to evaluate the performance (relevance and reliability) of the BCOP • Using an alternative corneal holder (e.g., holder developed by Ubels et al. 2002) • Using alternative vehicles for the test substance diluent (e.g., 0.9% NaCl) ICCVAM endorsed these studies as high priority activities.		
Pyrogen-icity	NIEHS	Ongoing	Validation Status of *In Vitro* Pyrogenicity Tests using Human Cells	Valid	NICEATM is supporting the ICCVAM Evaluation of f 5 *in vitro* pyrogenicity test methods using human cells intended as replacements for the rabbit pyrogen test by convening an International Independent Scientific Peer Review of the methods	Reduce Replace	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Repro-ductive/ Develop-mental	FDA	Ongoing	Development of High Throughput, In Vitro Systems for Identifying Potential Developmental Toxicants	Res Devel	Development of high throughput, mechanistically-based assays that can be used for prioritizing potential teratogens for further testing, elucidation of their mechanisms of action, and using mechanism of action information to determine if relationships exist between an agent's chemical structure and the potential to cause birth defects.	Replace	
Other (General toxicity testing)	FDA	Ongoing	Validation of the predictive performance of Caenorhabditis elegans (C. elegans) as a new animal model in toxicity testing and investigation of host-pathogen interactions	Valid	Validating short term toxicity assays utilizing growth, maturation, reproduction and survivability as endpoints of toxicity in *C. elegans*	Replace	
Other (Hepato-toxicity)	FDA	Ongoing	Validation of Short Term Assays for Hepatotoxicity	Res Devel Trans Valid	Development and validation of a liver cell-based culture system and relevant endpoint assays for evaluating the hepatotoxic potential of food-related chemicals and the virulence of hepatotrophic food borne microorganisms.	Reduce	
Other (Hepato-toxicity)	FDA	Near-future	Differential Gene Expression in Rodent and Human Primary Hepatocytes Exposed to the Peroxisome Proliferators Activated Receptor (PPAR) Alpha Agonists	Res	Identification of biomarkers and mechanisms of liver toxicity resulting from exposure to PPAR alpha antagonists using rodent and human primary hepatocytes.	Reduce	
Other (Biomarkers of Toxicity)	ATSDR	Ongoing	Use of biomarkers data (microarray) in computational models	Res Devel	Incorporation of biomarkers data to develop improved computational models (such as PBPK) for risk assessment	All 3	

Type of Toxicity Testing	Agency	Status[1]	Title	Type of Activity	Description	Potentially Applicable 3Rs	Other Information
Other (Biomarkers of Toxicity)	FDA	Near-future	Development of "Mitochip", a Glass-based Oligonucleotide Microarray Containing Mitochondrial and Nuclear Genes Associated with Mitochondrial Function	Res	Development and validation of a microarray system to detect genes associated with mitochondrial function as biomarkers of toxicity.	Reduce	
Other (Biomarkers of Toxicity)	FDA	Near-future	Development of a Novel Class Prediction Method, Decision Forest, for Analysis of Genomic and Proteomic Data	Res	Identification of biomarkers of toxicity associated with exposure to anesthetic agents by developing two-class and multi-class Decision Forest methods with gene expression and proteomic data sets..	Reduce	

[1]Refers to the timeframe for conduct of the ongoing or planned research activity